STUDY ON BIODEGRADATION OF SINGLE USE POLYTHENE USING SELECTIVE EARTHWORM SPECIES AND THEIR GUT-BACTERIA

SYNOPSIS

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Introduction

Plastic such as polythene is an important material used in infrastructure in modern society and its low cost, low weight, inertness as well as design versatility make it a choice at broad range having various applications (Andrady et al., 2015). Plastic pollution is among the biggest environmental challenge whereas it was expected that production of plastic may double in next few years which would focus on the key factors like recycling capabilities as well as waste management strategies (MacArthur et al., 2016). However, since last few years, plastic production has been tripled at the global level (Feil and Pretz, 2020).

Thompson coined term microplastic in 2004 (Thompson et al., 2004). Plastic particles having size less than 5mm are regarded as microplastics. They are point of concern as they are known to cause damage to various ecosystems (Lwanga et al., 2017).

Microplastics released as toxic contaminants into the water bodies as well as soils which then act as pollutants and cause harm to the environment (Wang et al., 2019) as they are capable of entering into number of food chains leading to different health problems (Lwanga et al., 2017).

Undesirable accumulation of plastics in landfills causes leaching into soil and an increase in green house emission which affects soil microbiota affecting terrestrial life (Lahive et al., 2019; Selonen et al., 2020).

Biodegradable polythene is capable of degrading when subjected to suitable environmental conditions, which are essential for waste management (Gouda et al., 2012). Biodegradable plastic production at global level contributes to about 1% of total plastic production which accounts for about 4 million tons annually estimating about 380 million tons (Geyer et al., 2017). Estimates show that around 8.3 billion of plastics being generated is treated as single-use product and have been discarded under natural environmental conditions (Nielsen et al., 2020).

The exponential increase in the use of plastic has given rise to growing concern of people including government and media (Geyer et al., 2017) as a wicked multiplicity of challenges are represented by the global plastic crisis (Landon-Lane, 2018). Pollution due to microplastics is known to be the main cause of concern in relation to ecology as well as material science. Various studies as well as approaches would be beneficial to understand the nature and process of microplastic biodegradation.

Earthworms are known to promote the efficiency of the biodegradation process due to their impact with regard to the micro-biota present in the surrounding soils (Brown et al., 2004). They are capable of altering biological as well as the physicochemical properties of the soil (Brown et al., 2000) which also affects growth of the plants (Scheu, 2003). Presence of earthworms is a indicator of soil health (Gavinelli et al., 2018). Feeding and casting of earthworms causes the promotion of soil nutrient cycles that contributes in enhancing soil fertility (Van Groenigen et al., 2019).

Earthworms possess variety of microbes including various bacterial and fungal strains in its gut micro flora. Likewise, micro- organisms occurring in the surrounding soils also show an important role affecting the terrestrial ecosystems which constitutes of the geo-chemical cycles, degradation of the pollutants along with the various number of adaptive changes which influence the environment (Murugan et al., 2014).

Process of plastic biodegradation is also influenced by the gastrointestinal lumen of the earthworms as its gut act as an inhabitant with high carbon and nitrogen content if compared with the surroundings which insist gut to be capable of anaerobic microbe colonization (Pass et al., 2015).

Literature Review

Soil is an essential asset for the farmers because all the activities related with agriculture occurs invariably in the soil. Long term stability as well as productivity are determined by the biological properties of the soil with regard to the sustainable management. It is known that soil organisms contribute in enhancing the soil productivity, however small- scale farmers are not much aware of organisms residing in the soil habitat, which are capable of affecting the properties of terrestrial ecosystem. Earthworms are very essential to farmers as they play magnificant action in the soil (Ebewore & Ovharhe, 2016).

Earthworms have been distributed throughout the world which portrays various essential activities playing an essential eco- functional role in terrestrial ecosystem influencing physical, chemical and biological characteristic features along with the productivity of the soil.

Different studies have been done in relation to the organisms present in the soil along with their contribution towards sustainable ecosystem (Barrios, 2007).

Earthworms modify litter and soil environment invariably by the accumulation of their biogenic structures. Cycling of nutrients in an ecosystem act as carping function that is important for the life present upon the earth. They are most beneficial agents of organic

waste products whose disposal may otherwise result into serious consequences for the maintenance of clean and healthy environment. The biodiversity of earthworm species has become one of the essential lacunas to work out at different areas due to their role in organic farming, sustainable agriculture, waste management and environmental conservation (Agarwal, 2005; Ranganathan, 2006).

The biomass constituting of soil macro- invertebrates such as earthworms may cause increase in the human as well as livestock biomass (Pimental, 2002). Higher and bountiful diversity of earthworm species across various types of environments shows that earthworms act as sensitive indicators in response to the land use (Paoletti, 1999; Lee et al., 2000).

Earthworms are known to be the indicators of agro-ecosystems as land belonging to agricultural activities is intensively occupied by them. Earthworms are the invertebrates which are known to perform a major action in the organic matter recycling existing in the soil. A wide range of enzymes and microbes are harbored inside earthworms gut micro-flora which help in the biodegradation process.

Diversity of earthworms in the world

Earthworms have been widely distributed mainly in the temperate as well as tropical areas of the world moreover, their population constitutes about 80% of the total biomass constituting soil (Nainawat & Nagendra, 2001).

Earthworm distribution is irregular and their number varies according to the type of soil along with the ecological factors mainly the edaphic factors (Chaudhuri et al., 2016).

The study of earthworm's biodiversity has been documented by a number of biologists at various parts of the world. More than 4200 Oligochaetes species are reported from the world and out of them 280 belong to the Microdii group whereas remaining 3200 belong to Megadrili group (Tripathi & Bhardwaj, 2001).

Worldwide, the identification of approximately 4,400 species of earthworm has been done (Sinha et al., 2009) and specific work on earthworms has been reported from the countries like New Zealand (Lee et al., 2000), France (Bouche, 1972), Canada (Reynolds, 2000), Australia (Jamieson, 1981), British Isles (Sims and Gerard, 1985), Pakistan (Rana et al., 2002), Libiya (Nair et al., 2005), Taiwan (Blakemore et al., 2006), Brazil (Sautter et al., 2006) and Thailand (Soomniyam & Suwanwaree, 2009).

Diversity of earthworms in India

India is known to be a diversified country accommodating a rich and varied number of earthworm species, which is generally constricted into the western ghats as well as

Himalayas in the east, that are being regarded as the hotspots of the biodiversity. However, this area just covers about 2% land mass of the world, supporting approximately 105% of the earthworm's diversity, which is estimated to be around four thousand species. India is rich in earthworm resources constituting of both peregrine and endemic species. Earthworms have been broadly distributed in India. Native species mainly contributes into the earthworm's faunal diversity of India that includes around 89 per cent of earthworm's diversity restricted to the country (Julka & Paliwal, 2005).

Higher molecular weight, hydrophobic nature along with 3-D structure shows intractable nature of the polymers which may be distorted showing impact on its availability towards microbes (Hadad et al., 2005).

Since last few decades, use of plastics has been increased on a large scale in different sectors including industry, transportation, packaging and agriculture which has led to the serious issue of waste disposal and pollution in rural as well as urban areas. It is intractable for biodegradation process and thus quite tough to get degraded under natural conditions. Plastic use has been grown at the rate of 12% per annuum whereas about 0.15 billion-ton synthetic polymers are being generated yearly at the global level (Leja & Lewandowicz 2010).

Plastic accumulation efficiency rate under various environmental conditions is about 25 million ton annually which has been regarded as a dangerous entity for the environment (Sivan et al. 2006; Thompson 2004).

Various parameters include temperature, pH, stress, moisture, sun- rays, H_2O, aerobic, anerobic as well as culture conditions that are known to affect the process of degradation as they exhibit a strong impact upon the microbe colonies and enzymes associated with the process (Gu, 2003).

Research Gap Identification

Waste management and degradation of plastic is a burning issue as well as need of an hour. At present there no effective and eco-friendly ways to get rid of plastic accumulation in the surroundings. The process is slow and thus plastic tends to persist in the environment for very longer durations which result in many harmful effects affecting flora and fauna. According to literature some earthworm species have potential to cause degradation as their gut micro-flora inhabits certain microbes including bacteria and fungi. However, there is very less literature so far with regard to this issue which is acting as a major research gap. Maximum studies as per literature emphasis on the toxicological effects

whereas very less attention is being given towards the solution resulting in efficiency as well as potential of earthworm species for remediation of pollutants like plastics. Hope that proposed work would be beneficial to the environment and society as its uniqueness may open more scopes for the future research with reference to this context.

Objectives

1. To access the efficiency of selected earthworm species in microplastic degradation
2. To investigate the potential of earthworm's gut bacteria for microplastic degradation
3. To access the impact of bioaugmentation in vermicast (containing microplastic) influencing plastic biodegradation process

Proposed Methodology

Earthworm species will be cultured in order to find out their potential for plastic degradation process. Particles having size less than 5mm are considered as microplastics thus chopped and fine particles of various types of plastics will be used. The microplastics or nano-plastics will be subjected to the different epegic earthworm species in order to get best species having degradation potential. Gut-flora of best selected best species will be studied. Bacterial strains will be studied isolated and cultured. Their strains will be subjected to microplastic concentrations and their potential for degradation will be checked. Strain showing best results will be selected. After that, final setup called bioaugmentation will include culturing of selected earthworm species and selected bacterial strain together along with microplastic exposure to give out the more promising results with context to the microplastic degradation process.

Different abiotic as well as biotic conditions will also be studied and standardized in order to get the best results. Abiotic conditions include temperature, pH, moisture, oxygen level, sunlight and UV rays whereas biotic conditions will include various microbes like bacteria inhabiting earthworm's gut micro-flora and the surrounding soil.

1. Screening of earthworm species having potential and efficiency for degradation process:

1.1 Collection, identification and selection of the earthworm species

Different earthworm species will be taken from different areas and will be identified. Out of them, epigeic species will be selected and cultured as they are known to increase and enhance bacterial diversity which is helpful in polythene degradation (Gopal et al., 2017).

1.2 Culturing the selected species under natural conditions

Different replicas of each earthworm species will be cultured in the laboratory and will be subjected to the natural conditions. Cow dung, soil and litter will be added in a proper quantity and conditions like moisture, pH and temperature will also be recorded time to time (Rodriguez et al., 2019).

1.3 Subjecting earthworm cultures microplastics

Polyethylene microplastics will be used. Earthworm species will be subjected to microplastics and degradation rate will be checked (Lwanga et al., 2017).

1.4 Analysis of the degradation process

Changes occurring in the chemical properties would be analyzed by the formation and disappearance of functional groups using FTIR and changes in the molecular weight distribution will be marked by TLC (Arutchelvi et al., 2008).

Now as per result analysis, screening will be done, in order to find out the species giving best results.

2. Screening of bacteria having potential and efficiency for degradation process

2.1 Isolation of bacteria from earthworm's gut

Earthworm's gut microflora has useful microbes consisting of bacteria and fungi. Bacteria will be studied and isolated from earthworm's gut. Selected earthworm species exposed to microplastics (Lwanga et al., 2016) will be collected, rinsed with ethanol and dried. Then it will be frozen under cold and sterilized conditions. After that dissection will be done in order to expose and extract its gut epithelium under sterile conditions (Garbeva & de Boer, 2009).

2.2 Preparation of culture media and serial solutions

Isolated bacteria will be allowed to grow in a culture media, Nutrient Agar. Preparation of culture media and preparation of serial dilutions will be done for the growth and identification of bacterial isolates.

2.3 Identification

2.3.1 Morphological characterization

It will include the study of morphological characters like colony size, colony shape, colony pigmentation.

2.3.2 Gram staining of the bacteria

It will be done do classify bacterial isolates as Gram positive and Gram negative respectively.

2.3.3 Biochemical Characterization

It will include:

Indole production test: It shows the presence and absence of tryptophanase.

Methyl Red test: It is used for the detection of acid end products.

Citrate utilization test: It is used for the detection of bacteria capable of utilizing Sodium citrate as a carbon source.

Catalase test: Catalase is produced by the bacteria that respire using oxygen.

Analysis will be done as per the outcome, and different bacteria confirmed will be taken and cultured separately along with exposure to the microplastics.

2.4 Isolated bacterial isolates will be cultured along with an exposure to microplastics

Selected bacterial isolates will be cultured and studied to check out the degradation potential. At last, testing will be done to check out the results and on the basis of that bacteria showing higher potential for degradation will be selected (Lwanga et al., 2018).

2.5 Testing and screening of bacteria having better potential for plastic degradation

Along with FTIR, various biological tests especially Clear-zone test would be carried for screening microbes having degradation potential (Abou-zeid et al., 2001 and Arutchelvi et al., 2008). Strum test would be done for estimating CO_2 evolution whereas Respirometry test would be done for estimating oxygen consumption (Hoffmann et al., 1997).

3. Final setup for bioaugmentation process consisting of selected earthworm species and bacteria

The best earthworm species having higher polyethene degradation potential will be cultured along with the selected bacteria and will be exposed to the polyethene microplastics. This process is also referred to as bioaugmentation which may show more promising results with context to the biodegradation process (Sharma & Jain, 2020). Testing will be done and results will be analyzed and compared.

Thesis Outline

Thesis will be constituting six chapters. Namely, they are as follows:

Chapter 1 (Introduction): It will be including the general information and objectives. Definition, types, generation and status of microplastics in the environment along with their harmful effects. Various selective species of earthworms and their role in the degradation process will also be stated in it.

Chapter 2 (Literature Review): It will include the brief and deep content in context to the topic which will constitute the old as well as new findings stated in the literature so far. It will also include research gap identification.

Chapter 3 (Materials and Methods): It will contain detailed information about the species selection, culture maintenance, conditions, field and lab reports.

Chapter 4 (Results and Discussion): This chapter will be containing the data regarding various changes occurring in the polymer along with the degradation time. Comparison studies will be done with reference to the exposure of the cultures in natural as well as artificial conditions with context to the results noted.

Chapter 5 (Summary and Conclusion): This chapter will be comprising the conclusion of whole study based upon the objectives along with the summary. It will also include the discussion regarding future research scope.

Chapter 6 (References): This chapter will include all the reference used and stated in the thesis. The format of reference citation will be strictly according to the guidelines stated as per Harvard referencing style.

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CERTIFICATE
(SYNOPSIS APPROVAL CERTIFICATE)

This is to certify that the Synopsis for Ph.D. Thesis titled, **"STUDY ON BIODEGRADATION OF SINGLE USE POLYTHENE USING SELECTIVE EARTHWORM SPECIES AND THEIR GUT-BACTERIA"**, submitted by **Ms. Aishwarya Sharma** to the Career Point University, Hamirpur (H.P.), India in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in the Division of **Zoology**, Department of **Biosciences**, School of **Basic and Applied Sciences** has been approved by the Research Advisory Committee (RAC) after an Oral Examination of the student on dated 01.06.2022.

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